THE KATAGIRI LABORATORY

AUTHOR(S):

Katagiri, Hideo

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Head: Prof. Dr. Hideo Katagiri

The Katagiri Laboratory was established in March 14th, 1942, under the directorship of Hideo Katagiri, Professor of Physiology of Fermentation and Brewing in the Department of Agriculture at Kyoto University, and the following experimental results were obtained in the Laboratory during nine years.

1. Studies on Alcoholization of Fiber Materials

Saccharification of various kinds of fibre materials, including wood and barks of Mulberry tree, cotton plant, bog-moss in the thundra of Saghalien, rice and barley straws, rice hulls etc., was carried by dilute sulphuric acid under pressure with an apparatus of Scholler's type, which was planned by Dr. Masuzo Shikata, ex-professor of the Institute.

It was found that saccharification of fibre materials, especially of bog-moss is well achieved, if they are removed of humic acid beforehand with dilute ammonia.

Among various strains of yeasts, a kind of distillery yeast suitable for fermentation of sacharified solutions was selected, and no inhibiting substance to the yeast was detected, though 0.82 g of furfural, 0.021 g of hydroxymethylfurfural and 3.25 g of volatile acids including formic, acetic and levulinic acids, were produced by decomposition of 100 g of Mulberry tree in the saccharified solutions.

Thus, in decomposing 100 g of bog-moss by dilute acid at 150°C, about 30 g of sugar was obtained and then about 9 g of alcohol was produced by fermentation of saccharified solution. About 33 g of sugar and then about 10 g of alcohol were obtained by decomposition of 100 g of Mulberry tree with dilute acid at 180°C.

Spent wash (pH 5.5), distillery waste liquor of the alcohol solution obtained from saccharified solution of Mulberry tree was found to contain 2.043 g of organic matters, 0.993 g of reducing sugar (as glucose), 0.470 g of pentose and 0.084 g of total nitrogen in 100 cc. In order to find out availability of these constituents to yeasts, with three strains of pentose-assimilation yeast (named Torulopsis xylinus

Table I. Crop yield of yeast from spent wash

Strains	Period of cultivation							
	12 hrs		18 hrs		24 hrs		36 hrs	
	Weight of yeast g/100 cc	Remaining sugar g/100 cc	Weight of yeast g/100 cc	Remaining sugar g/100 cc	Weight of yeast g/100 cc	Remaining sugar g/100 cc	Weight of yeast g/100 cc	Assimilation of sugar %
T. xylinus a	0.085	0.863	0.190	0.781	0.240	0.692	0.267	41.58
b	0.135	0.833	0.159	0.817	0.249	0.682	0.253	39.31
c	0.200	0.821	0.273	0.755	0.285	0.648	0.271	49.49
T. utilis	0.136	0.843	0.229	0.763	0.265	0.636	0.244	44.98

a, b and c) isolated by us and with Torula utilis previously acclimatized to pentose, experiments were carried out with spent wash having been added Reader's nutrients.

During 120 hours' ordinary culture, 1/3 parts of reducing sugar, including more than 2/3 parts of pentose, were consumed. In the case of shaking-culture (see Table I), the yield of yeast, especially newly isolated yeast Torulopsis xylinus c, attained the maximum as a result of cultivation for 24 hours, when 41~46%, 30% and 75% yields of yeast were observed to organic matters, total nitrogen and to pentose respectively, therefore 9.5 g of dried yeast was obtained from 100 g of bog-moss.

It will be seen in Table II that these Torulopsis were again found to grow remarkably by means of shaking culture in sweet potato juice (containing 2.87 g of reducing sugar, 5.50 g of total sugar, 0.15 g of total nitrogen, 61.4 γ of vitamin B_1 and 41.4 γ of vitamin B_2 in 100 cc), of which pH was adjusted to 4.5 by HCl, then kept for one hour at 70°C, and filtered.

No remarkable multiplication of yeasts took place with sulphite pulp liquor (containing 14.05 g of total solid matters, 0.99 g of ash, 0.012 g of nitrogen, 3.235 g of sugar as glucose, 0.453 g of lignin and 0.1984 g free SO_2 in 100 cc) from which half of SO_2 was expelled by aeration for 20 hours and the remaining SO_2 was precipitated by adding calcium hydroxide to pH 7.0, filtered after having been heated 100°C for 30 minutes, and then adjusted pH to 5.6 by sulphuric acid, while noticeable crop yields of the yeasts, especially of Torulopsis c, were obtained, when 0.2 g of ammonium sulphate and 0.1 g of potassium phosphate were added to 100 cc of the SO_2 free sulphite pulp liquor.

Table II. Crop yield of Torulopsis from sweet potato juice and sulphite pulp liquor

Cultural solutions	Strains	Period of cultivation			
		3 hrs	12 hrs	24 hrs	
		Weight of yeast g/100 cc	Weight of yeast g/100 cc	Weight of yeast g/100 cc	Assimilation of sugar %
Sweet potato juice	a	0.161	0.457	1.026	88.20
	b	0.175	0.448	0.932	84.20
	c	0.190	0.540	1.001	88.44
Sulphite pulp liquor added $(NH_4)_2SO_4$ and KH_2PO_4	a	0.066	0.127	0.198	49.57
	b	0.061	0.129	0.253	45.43
	c	0.088	0.205	0.392	49.57

The chemical compositions of yeasts Torulopsis (7~9% of ash, 43~47% of crude protein and 0.69~1.1 mg% of vitamin B_1) thus obtained were found to be very much the same with Torula utilis obtained from spent washes of ordinary wood or pulp waste liquor.

Relating to the microbiological utilization of pentoses, experiments were instituted to produce antibiotics by Pseudomonas aeruginosa and to obtain propionic acid by pentose assimilating bacteria, Propionibacterium zeae, P. arabinosum, P. pentosaceum and Propionicoccus japonicum isolated by us.

2. Application of the Method of Fermentation-Retting

Fermentation degumming of waste silk and then fermentation retting of various kinds of plant fibre materials, including ramie, hemp, flax, kenaf, jute etc., were investigated by us, and we succeeded in the isolation of useful retting-bacteria and also in the industrialization of fermentation-retting of these fibre materials (Patents No. 160920 in January, 1944 and No. 167483, October, 1944).

In order to test whether the degree of degumming by fermentation-retting varied from different sections of a ramie plant, or whether the alkaline treatment would reveal any noticeable effect on refined fibres by fermentation, experiments were carried out with ramie plant cultivated in Shimane prefecture where it was decorticated with a decorticating machine.

For physical examinations, the fibre taken from each of several selected sections of refined fibres, and for the chemical examinations, the fibre taken from the same sections respectively, of refined and raw fibres were employed. These sections were as follows:

A. Root... lower 6 inches of a ramie plant.

B. A 6 inch section removed at a distance equal to 1/3 of the length of a ramie plant measuring from the root tip.

C. A 6 inch section removed at a distance equal to 2/3 of the length of a ramie plant measuring from the root tip.

D. Top... upper 6 inches of a ramie plant.

In different sections of raw fibre material, chemical compositions differed greatly; the root section (A) contained a greater amount of cellulose and the least amount of ash and pectin, while the least amount of cellulose and a greater amount of lignin, ash, nitrogen, pectin and pentosan were found in the top section (D). The effect of fermentation retting was found satisfactory in all sections of the ramie plant, since the amount of pectin decreased less than 0.65 % and the amount of cellulose increased to 92.48~94.70% by 70-hour fermentation as seen in Table III.

Useful effect of alkaline treatment (boiled for one hour in NaOH solution in

Table III. Chemical composition of ramie fibre

Materials		Moisture (%)	% of bone dry fibre									
			Extractable matters by			Cellulose	Lignin	Pentosan	Methoxyl	Pectin (Ca-salt)	Nitrogen	Ash
Treatment	Section		Alcohol	Ether	Hot water							
Raw fibre	A (Root)	12.16	3.82	6.64	8.22	81.98	3.33	5.09	0.43	3.81	0.50	1.37
	B	12.35	3.96	6.47	7.48	83.63	3.12	6.85	0.32	4.27	0.48	1.13
	C	12.57	4.67	6.19	9.48	79.73	3.40	6.53	0.44	4.55	0.69	2.37
	D (Top)	13.73	4.71	5.74	12.70	68.83	4.59	6.87	0.53	5.29	1.10	4.34
Fermentation only	A (Root)	10.33	0.90	5.13	0.94	92.48	2.87	2.90	0.22	0.65	0.29	0.83
	B	10.14	0.87	5.56	0.84	94.20	2.15	2.24	0.19	0.55	0.23	0.55
	C	10.13	0.88	3.94	0.74	94.70	2.81	2.07	0.18	0.60	0.32	0.77
	D (Top)	9.93	1.59	5.04	1.06	94.00	3.31	2.54	0.19	0.61	0.45	1.26

which 2 % of NaOH to fibre material existed) with fermented fibre material of high class was found neither in chemical compositions nor in the physical natures (Denier, strength etc.). Such an alkaline treatment could probably applicable to shorten the fermentation period for the lower class of fibre materials, although not only the quality, but also the yield dropped to 75.5 % from 81 % by alkaline treatment in contrast with the case of high class fibre materials in which the yield of refined fibre (85.5 %) was not altered by alkaline treatment.

Further experiments were carried out in order to test whether the theory proposed by us, in which the specificity of fermentation-retting of plant fibre materials was attributed to the nature of protopectinase produced by retting bacteria as shown in Table IV, will be applicable to other bacteria relating to fermentation retting.

Table IV. Specificity of Protopectinase of retting bacteria

Protopectinase	% of decomposition of protopectin prepared from fibre materials				
Bacteria reveal selective action on fibre materials	ramie	hemp	flax	kenaf	jute
Bacillus subtilis, on ramie	35	28	15	4	2
Micrococcus cannabis, on hemp	19	40	25	12	1
Micrococcus linumus, on flaxs	11	30	35	9	29
Micrococcus hibiscus, on kenaf	18	8	12	39	22
Bacillus corchorus, on jute	30	24	5	11	42

It was verified that the strains of retting bacteria for degumming various kinds of plant fibre materials including Abelmoschus glutinotextilis, barks of Mulberry tree and barks of Ricinus communis, etc., also revealed the specific nature expected under our theory.

In order to test how much degree of degumming could be attainable for barks of Mulberry tree, experiments were carried out with Bacillus morus isolated by us. Proportional to the period of fermentation, the amount of α-cellulose increased; 61.82, 69.77 and 76.39 % α-cellulose containing refined fibres were obtained by 50-168- and 360-hour fermentations, respectively.

A new retting bacteria named Bacillus ricinus was isolated from barks of Ricinus communis. As the results of 72- and 200-hour fermentation of bark, with bacteria, refined fibres, containing 82.87 and 84.63% of α-cellulose, were obtained.

With the bleaching powder treatment of these refined fibres fermented nearly 60 hours, α-cellulose increased to 90.65%. Consequently, fermentation retting seems to be useful method for degumming the barks which serves as material for Japanese paper.

Application of the method of fermentation retting was not limited to fibre materials, since bacterial fermentation was found to be applicable to the process of purification of B-seed lac, i.e., the waste material in the manufacture of shellac. Of 40 strains of bacteria isolated by us, one strain of cocci, which was ascertained to belong to Pseudomonas myxogenes Fuhrmann, was found to be the

most useful bacteria. The successful results in which the nitrogen substances, including alcohol soluble protein in the B seed lac, were attacked greatly by Pseudomonas for 5-day fermentation, were obtained, and the continuous fermentation process, which was proposed by us (Patent No. 167483) in the fermentation retting of plant fibre materials, was again found to be applicable to the bacterial purification of B seed lac.

It is noticeable that the retting bacteria, Bacillus subtilis var. employed to the fermentation of ramie fibre materials, reveals antibacterial action on Staphylococcus aureus, Escherichia coli and eleven strains of bacteria among 14 strains isolated from a naturally fermented solution composed of peptone water and wild ramie plant, and, accordingly, the fermentation retting with B. subtilis var. goes on smoothly without any troublesome contamination even if the retting process is carried out with open vessel.

3. Studies on Iron Bacteria and Sulphur Bacteria which would reveal Inhibiting Effects on the Synthesis of Ammonia

The isolation of iron bacteria which cause the corrosion of the coal gas washing tower was successful. These bacteria were found to belong to Leptothrix ochracea which was ascertained to exist widely throughout Japan. It was concluded that corrosion caused by these iron bacteria will be checked by keeping pH of the tower water over than 8.0 or lower than 4.3, in accordance with the result of our investigation of cultural conditions (the optimum pH was 5.5~6.0, the limiting pH was 8.0 or 4.3, the optimum temperature was 20~25° and the limiting temperature was 2.5° or 41.5°C) of these bacteria.

The sulphur bacteria producing hydrogen sulphide were isolated from gas tanks of ammonia factories. These sulphur bacteria were determined to belong to Microspira desulfuricans, the existence of which were again found to be located throughout Japan. The cultural conditions of these bacteria were found as follows: the optimum pH was 6.1~8.5, the limiting pH was 9.6 or 5.5, the optimum temperature was 25~28° and the death point was 55°C for 30 minutes and the multiplication of these bacteria in the gas tank was inhibited by keeping pH of the sealed water lower than 4.3.

4. On the Manufacture of Diastase

The ordinary process of the manufacture of diastase from malt or koji was followed by precipitation with alcohol from the aqueous extract of the materials, and therefore, a large amount of impurities were found in the diastase preparations, though the abundant quantity of alcohol is employed.

In order to get not only a highly active preparation of diastase but also to shorten (about to 1/2) the usage of alcohol, a new method was invented (Patent No. 126296, August, 1938) in which diastase was extracted with dilute potassium sulphate solution to dissolve even zymogen forms of enzyme, and the enzyme was precipitated by tannic acid followed by washing away the tannic acid combined with the precipitate by acetone or alcohol. For industrialization of our patented method, series of experiments were carried out.

In order to precipitate the enzyme from the solution obtained by extracting

dried malt with 1 % solution of potassium sulphate, 5 % solution of tannic acid was added to the enzyme solution, adjusting pH to 5.3. The tannin precipitate was separated by centrifuge, washed with cold water several times, and then the fresh precipitate was washed with acetone or 80 % alcohol solution of pH 6.2 adjusted by sodium acetate, and again centrifuged in order to collect the enzyme preparation.

It was found that the activity and yield of enzyme to be superior to the ordinary preparation (IV) even the precipitate was desiccated under reduced pressure on sulphuric acid at ordinary temperature (II) as shown in Table V in which preparation (I) represents the crude extract of dried malt with potassium sulphate.

Table V. Yield and activity of diastase preparations

Preparation	Yield %	Saccharifying power		Starch liquefying power	Proteoclastic power
		α-Amylase	β-Amylase		
I	100	0.12	1.00	1.0	1.0
II	59	6.24	48.13	42.7	29.3
III	50	8.64	85.36	64.0	58.2
IV	54	5.25	41.26	45.7	19.4

When the enzyme preparation was dissolved in water in order to separate insoluble matters and dried under reduced pressure (1/100~2/100 mm.Hg) with freezing trap of −70°C, a white and easily soluble preparation (III) was obtained.

It will be seen that, in the above Table, the highest powers of these enzymes are found in preparation (III), and preparation (II) reveals higher enzymatic powers except liquefying power, when it is compared with preparation (IV). No remarkable difference in the ratio of saccharifying power to liquefying power was observed among all these preparations, but proteoclastic power was found to be diminished by the treatment of precipitation, especially with alcohol.

It is interesting to note that the same tannic acid recovered from acetone or alcohol solution can be employed repeatedly so many times.